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REVIEW ARTICLE

Detection of Occult Metastases in Lung Carcinomas: Progress and Implications for Lung Cancer Staging

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The ability to detect occult regional and systemic metastases in patients with operable lung carcinoma could have a significant impact on the management of the disease. Here, we review the literature, including studies from our own laboratory, regarding the clinical significance of the presence of occult metastases in patients with lung cancer. The accumulated evidence strongly suggests that the detection of occult regional and systemic metastases is an important predictor of disease progression. The use of this method should be considered in the future design of lung cancer clinical trials, at the very least. The detection of occult metastases should have an impact on lung cancer mangement; to reflect this, we propose a change in the TNM staging system to indicate the presence or absence of occult regional (lymph node) and systemic (bone marrow) metastases. The proposed change is TNnMm, where *n* and *m* are occult nodal and bone marrow metastases status.

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KEY WORDS: **lung cancer; occult metastases; immunohistochemistry; bone marrow; lymph nodes**

INTRODUCTION

Lung cancer is the third most common form of cancer and the leading cause of cancer deaths in the United States among both men and women [1]. Approximately 178,100 new cases were diagnosed and 160,400 people died from this disease in 1997 [1]. The incidence of the disease in women continues to increase, and the death rate due to lung cancer has surpassed that for breast carcinoma. It is anticipated that lung cancer will continue to be a significant health-care problem for the foreseeable future.

Once a lung cancer has developed, surgery (either alone or in combination with adjuvant therapy) represents the most effective treatment for lung cancer [2]. Surgery is most effective in patients with stage I or II non-small cell carcinoma and is largely restricted to those who are able to tolerate the required pulmonary resection. The goal of surgery is to remove all possible tumor, both gross and microscopic. Of the four major histologic subtypes of lung cancer (squamous, adenocar-

This paper is dedicated to Dr. Edward J. (Ted) Beattie, Jr. (1918–1998). Mentor, colleague, and friend, he was a true giant in the fight against cancer.

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cinoma, small cell, large cell), only small cell carcinoma is generally considered refractory to surgical therapy. However, small cell carcinoma accounts for only 22% of lung tumors overall [3]; thus, 78% of lung carcinomas would be potentially curable by surgery if they were detected early enough. Approximately 50% of patients with lung carcinoma are candidates for, and will undergo, definitive surgical resection [2].

Lung Cancer Staging

Several factors influence the outcome of patients with lung cancer, including the stage of disease, histologic type, degree of differentiation, status of various biologic prognostic markers including DNA ploidy, the proliferative fraction of the tumor (i.e., the percentage of tumor cells in S phase), oncogene amplification, and tumor-suppressor gene alterations including c-myc, Her 2/neu, p53, and retinoblastoma (Rb). Still, the most important factor in predicting outcome and defining therapy is the stage of the disease. In particular, the TNM staging system and the staging map of the mediastinal lymph nodes [4] have been very helpful in establishing treatment plans and determining prognosis for resectable lung cancer [5–8]. For non-small cell carcinomas, accurate staging of disease has greater prognostic significance than does cell type. For a T1, N0, M0 (stage I) lung carcinoma treated with surgical excision, preferably lobectomy, patients have an anticipated 5-year survival rate of 60% to 85% [5–9]. For larger carcinomas, such as T2, N0, M0 (stage I), patients have a 50% to 60% 5-year survival rate. Still, survival rates decrease dramatically with increasing stage of disease. In patients with stage III lung cancer, survival rates as low as 5% have been reported despite the absence of clinically detectable systemic metastases at the time of surgery [2].

Of the standard staging parameters, the presence or absence of lymph node involvement is perhaps the most important. Mountain [10] and Naruke et al. [11] have shown that the presence or absence of lymph node involvement has a major impact on the survival rates of patients with non-small cell lung cancer after surgical resection. The 5-year survival rate dropped from 57.2% in patients with node-negative disease to 37.2% in patients with metastasis to lymph nodes in the peribronchial and/or ipsilateral hilar region (N1). Survival rates dropped to 28.8% in patients with lymph node metastases to ipsilateral mediastinal lymph nodes and subcarinal nodes (N2) [10].

Role of Detecting Occult Metastases in Patients With Lung Cancer

Although traditional staging has been very useful in predicting patient outcome, the single most important determinant of prognosis and management of lung cancer is the presence or absence of occult metastatic dissemination of cancer at the time of initial presentation and treatment. This is because primary treatment failure is secondary to undetectable systemic spread of tumor. Current measures of disease extent are primitive; standard prognostic indices, although providing reliable information about which populations of patients will experience disease recurrence, cannot predict which individual patients will progress after primary therapy, particularly in the case of low-stage (I or II) disease. Furthermore, the success of adjuvant therapy is assumed to stem from its ability to eradicate occult metastases before they have become clinically evident [12].

The ability to detect the earliest systemic spread of lung cancer would identify several important groups of patients, including those with low-stage (stage I) disease who have evidence of occult tumor metastases and who may therefore benefit from more aggressive (systemic) treatment. In addition, patients with locally advanced (stage III) disease, who generally are not considered to be surgical candidates, may be identified and, thus, may benefit from more aggressive local (surgical) control of their tumor. Selected patients with stage III disease may be candidates for surgery if there is a reasonable expectation of total resection of all known tumor. Furthermore, the presence of occult metastases in the bone marrow might identify patients with small cell lung cancer who could benefit from surgical intervention. Finally, because the success of adjuvant therapy is assumed to stem from its ability to eradicate occult metastases before they become clinically evident, the detection of occult tumor could identify those patients most (and least) likely to benefit from such therapy. Therefore, the detection of occult metastases in the bone marrow could have substantial clinical impact not only in establishing prognosis but also in making the optimal therapeutic decision for patients with lung cancer.

TECHNIQUES FOR DETECTING OCCULT METASTASES

Recently, significant advancements have been made in the use of sophisticated technologies (immunohistochemical and molecular) to detect occult metastases in patients with the earliest stages of cancer, i.e., prior to the detection of metastases by any other clinical or pathologic analysis. Following pioneering studies at the Ludwig Institute and Royal Marsden Hospital in London [13], a number of groups have identified occult metastatic carcinoma cells in the bone marrow and lymph nodes of patients with cancer [14–22]. Although many of these studies have focused on breast carcinoma, many other tumors, including colon, prostate, and lung, are now under investigation [23–32]. The procedures used in these studies take advantage of the ability to distinguish between cells of different histogenesis, in this case cells

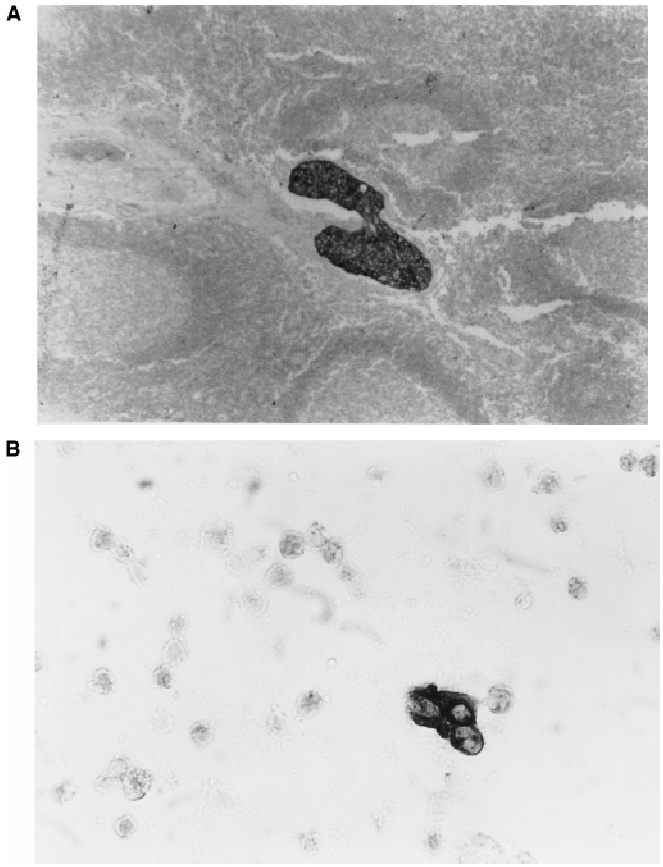

Fig. 1. **A:** Regional lymph node from a patient with occult lymph node metastases stained immunohistochemically with anticytokeratin monoclonal antibodies (AE-1 and CAM 5.2). **B:** Immunohistochemical staining with monoclonal antibodies to cytokeratin (AE-1 and CAM 5.2), showing a cluster of cancer cells in the bone marrow of a patient with lung cancer.

of hematopoietic vs. epithelial origin. The results indicate that it is possible to identify occult metastatic carcinoma cells in lymph nodes and in bone marrow prior to their detection by any other method (Fig. 1) and that the presence of these cells may be an important risk factor for disease recurrence.

Molecular Methods

Recently, molecular methods have been used to detect occult metastatic populations of cells. Because consistent abnormalities at the DNA level have not been identified for most epithelial malignancies (including lung cancer), the method usually employed is the reverse-transcriptase polymerase chain reaction (RT-PCR), which differentiates gene expression between epithelial and hematopoietic cells to identify epithelial cancer cells. Epithelium-specific RNA is reverse-transcribed to c-DNA and amplified by PCR using specific primers. This several thousandfold amplification of the signal theoretically makes the method extremely sensitive. However, the possibility of low-level gene expression of epithelium-specific genes in lymphoid cells can result in high background, as will be further described. A further shortcoming of this method is the inability to employ morphologic criteria to confirm the presence of metastatic cancer cells.

The PCR techniques do have certain advantages over immunohistochemistry (IHC), including its technical ease. Interpretation of results may also be easier, and there may be a slight increase in sensitivity. The data reported by Zippelius et al. [33], however, do not support the common view that PCR analysis is generally more sensitive than IHC; this group found that tumor cell detection in the bone marrow of patients with prostate cancer was more frequent when IHC methods were used compared to prostate-specific antigen (PSA) RT-PCR.

A major drawback in the molecular assessment of lymph nodes for occult metastases is that the entire lymph node must be disaggregated in its fresh state in order to obtain the mRNA, thus negating the possibility of routine histologic analysis of the lymph node. Furthermore, it has been shown that often only one lymph node is positive [34]. This means that all lymph nodes would have to be disrupted, thereby making them unusable for any type of morphologic evaluation. By contrast, when evaluating lymph nodes for occult metastases by IHC, only a single section from the paraffin block is required.

Because the cytokeratins are the most ubiquitous and highly expressed of the epithelium specific genes, they have been the focus of many studies; cytokeratin 18 or 19 (CK-18, CK-19) mRNA expression has been the most frequent target for amplification to detect occult metastases, although a wide variety of epithelium-specific markers have been evaluated, including carcinoembryonic antigen (CEA), PSA, cytokeratin 20 (CK-20), gastrointestinal antigen 733.2 (GA-733.2), and mucin 1 (MUC-1) [35]. Datta et al. [36] used the CK-19 transcript to identify cancer cells in the bone marrow and peripheral blood of breast cancer patients. It is known that a pseudogene for CK-19 exists which shares a very high homology with the mature mRNA from the CK-19 gene [36]. Contamination of the pseudogene DNA in the RNA sample used for reverse-transcription can result in amplification of the pseudogene, giving rise to specific ''background'' bands in the negative controls. Furthermore, nonepithelial cells may have a low level of expression of true epithelial transcripts. Epithelial cells from the hands of laboratory workers or other exogenous sources can contaminate the specimens, requiring strict sterile techniques with the PCR method.

The specificity of RT-PCR methods may be the most important factor limiting their application in the detection of occult metastases. We have recently evaluated the potential of specific mRNA markers to detect micrometastases by RT-PCR and Southern blot analysis in frozen tissue [35]. We examined the specificity of CEA, CK-19, CK-20, GA-733.2, and MUC-1. We found that CK-20 was the only mRNA marker not detected by RT-PCR and

Southern blot analysis in the lymph nodes or blood from noncancer patients. Both the blood and lymph nodes from the noncancer patients expressed mRNA for CEA, CK-19, GA-733.2, and MUC-1 [35]. Zippelius et al. [33] had similar results; in a study of 53 normal (control) bone marrow aspirates, a series of epithelium-specific markers were consistently positive by RT-PCR methods, including epithelial glycoprotein-40 (EPG-40), desmoplakin-1, CEA, erb-B2, erb-B3, and CK-18. Only PSA was not detected in control bone marrow specimens by RT-PCR. Thus, for a large series of epithelial markers, the use of RT-PCR for the detection of occult metastases will be limited due to unacceptable background in normal controls.

Finally, in nonprostate epithelial tumors, there are no studies to date showing that molecular methods for detecting occult metastases are associated with patient outcome. This is an important consideration for patient selection, particularly in the context of clinical trials.

Flow-Cytometric Methods

A flow-cytometric assay has been recently developed to detect rare cancer cells in bone marrow and blood [37]. It has been reported to be extremely sensitive, with an ability to detect one positive cell in 10^6 blood cells in a model system; this is in contrast to all other reports on the sensitivity of flow cytometry [38]. One major disadvantage of most cytometric systems is the inability to morphologically characterize the cells constituting the ''positive'' events. However, by employing sophisticated cell-sorting technologies, in which the extrinsic cell population can be captured for subsequent morphologic evaluation, the rate of detection might be improved. These methods remain to be clinically validated.

IHC Methods

Currently, there appear to be no techniques that equal the IHC method for detection of occult metastases. All studies showing that patient outcome is associated with occult lymph node and bone marrow metastases have been performed using IHC methods [15,17–20,24,28,29, 32,39,40]. Thus, IHC is the only clinically validated method for detection of occult metastases. IHC methods take advantage of the specificity of monoclonal antibodies and their ability to distinguish (on the basis of differential antigen expression) between cells of different histogenesis—in this case, between cells of epithelial and hematopoietic origin.

Several different antibodies have been used to detect metastatic carcinoma cells in bone marrow and lymph nodes [13,15–19,31,32]. The distinguishing feature of these antibodies is that they are, for the most part, epithelium-specific and do not react with normal lymphoid cells present in the bone marrow and lymph nodes. None of the antibodies used in any study is specific for cancer, and all react with normal and malignant epithelial cells. However, they are useful because they can identify an extrinsic population of epithelial cells in the bone marrow and lymph nodes, where there are normally no epithelial elements.

The sensitivity of methods used to detect extrinsic cells in the bone marrow is an important issue. The concentration of such cells in the bone marrow of patients with early-stage cancer is presumably quite low. Such cells cannot be detected by routine imaging procedures, biochemical determinations, or even cytologic examination of the bone marrow. The reported sensitivity of detection using IHC methods ranges from one cell in 10^5 to two to five in 10^6 normal hematopoietic cells [41–43]. Thus, the IHC procedures for the detection of occult metastases in the bone marrow are exquisitely sensitive and reach the theoretical limits of sensitivity of the RT-PCR methods.

Using IHC methods, occult metastases have been detected in the bone marrow of patients with a variety of tumors, including colon [20], prostate [23,27], breast [32], and lung [24, 28] carcinomas. Studies are furthest advanced for breast carcinoma. Tumor cells were found in the bone marrow of 20% to 45% of patients with primary operable breast cancer [32,44–47] and in 20% to 70% of patients with metastatic disease [48]. All of the studies have demonstrated that occult metastatic spread of disease not detectable even by careful pathologic, clinical, biochemical, and radiologic examination can be detected in the bone marrow of a proportion of patients with early-stage breast carcinoma.

In general, bone marrow aspirations collected at the time of surgery have been used. Dearnaley et al. [16] found that the yield of detecting positive cells improved when aspirations from multiple sites were obtained. In our studies, aspirations were collected from both the anterior iliac crests and the sternum [32]. Visualization of antigen-positive cells has been performed using either light microscopic methods or indirect immunofluorescent methods. We originally employed indirect immunofluorescence rather than light microscopy in our studies for two reasons [15]. First, we believed it would be easier to locate small numbers of fluorescent cells against a dark background. Second, light microscopic methods were thought to be more difficult to interpret because of high background staining. However, we and others have used immunoperoxidase and immunoalkaline phosphatase methods in light-field microscopy with good results [13,19,24]. Light microscopic visualization methods have the advantages of providing a permanent record and allowing better cytologic evaluation of cells.

Automated Detection of Occult Metastases

While IHC methods are extremely sensitive, manual microscopic screening of rare positive (i.e., antigen-

TABLE I. Bone Marrow Occult Metastases in Non-Small Cell Lung Cancer

Group	Total number patients	Total BMM^{+a} number (percent)	Median F/U^{b} (months)	Prognostic significance^{c}
Cote et al. [24]	43	17 (40%)	13.60	$P = 0.0009$
Ohgami et al. [53]	39	15 (39%)	4.70	$P = 0.0083$
Pantel et al. [28]	82	18 (21.9%)	13.00	$P < 0.05$
Pantel et al. [52]	66	12^{d} (18%)	39.00	$P = 0.004$

[a]BMM^{+}, Occult metastases in the bone marrow.
[b]F/U, Follow-up.
[c]Prognostic significance, The presence of occult metastases in the bone marrow predicted disease-free survival in all studies.
[d]Greater than one tumor cell in bone marrow.

expressing) tumor cells is labor-intensive and time-consuming and requires much expertise. Automated slide screening may facilitate the routine use of IHC methods to detect occult metastases. Attempts have already been undertaken along this line with encouraging results [46,49]. Based on our previous research in the detection of occult metastases in the bone marrow [15,24,32,41, 45,47], we have recently tested the sensitivity, accuracy, and reproducibility of one such system (provided by Centocor, Malvern, PA, and ChromaVision, San Juan Capistrano, CA).

The system uses wavelength-specific laser technology to detect color differences between cells; this is the basis for the IHC detection of occult metastases (i.e., the tumor cells react with epithelium-specific antibodies and are detected by a specific chromogen color change). The system is controlled via a rapid-rate event detection software program which allows detection, quantitation, and location of ''positive'' events. A positive event is determined by previously programmed criteria based on specific instructions concerning size and color threshold.

All positive events, as identified by the system, are digitized and stored in memory. When the system has completed its review of a case (generally at least five slides per patient), the positive events are displayed as a montage on a high-resolution computer monitor. This montage is reviewed by the pathologist, who makes the final determination as to whether a ''positive'' event actually constitutes a tumor cell; the true positive events (tumor cells as determined by the pathologist) are then stored in memory and quantitated. Questionable cells can be located on the original slide for review under light microscopy. This prescreening by the system for potential ''positive'' events is a strength of the technology as it provides for the elimination of false-positive events.

We have compared this system to our standard, clinically validated, manual methods for detecting rare tumor cells in bone marrow and blood. Evaluation of the sensitivity, accuracy, and reproducibility of the automated system was based on a comparison of results obtained by manual vs. automated screening methods.

Our results indicate that the automated system is at least as sensitive as manual slide screening at detecting tumor cells, with a rate of detection approaching one tumor cell in 10^6 hematopoietic cells. In fact, using the automated system, we found that the probability of detecting even a single tumor cell on a slide is high [50]. Furthermore, we have now demonstrated that automated detection of occult metastases in the bone marrow of patients with operable lung cancer is comparable to that of manual screening methods in the context of a prospective clinical trial and identifies patients at increased risk for recurrence, independent of tumor stage [51]. Thus, automated screening of specimens appears to be a sensitive and clinically valid method for detecting occult metastases. It may be useful in evaluating occult metastases in future clinical trials, a concept our group is now pursuing.

DETECTION OF OCCULT METASTASES IN THE BONE MARROW OF PATIENTS WITH LUNG CANCER

Non-Small Cell Lung Cancer

Several recent studies have examined the presence of occult metastases in the bone marrow in operable non-small cell lung cancer (Table I). Using antibody to CK-18, Pantel et al. [28] tested bone marrow from the iliac crest of 82 patients for the presence of tumor cells and detected occult metastases in almost 22% of those with operable non-small cell lung cancer. When compared with established risk factors, the detection of occult metastases in the bone marrow was found to be significantly associated with size and histologic grade of the primary tumor. A correlation between occult metastases in the bone marrow and clinical outcome was found: 66.7% of the patients who had immunohistochemically detected tumor cells in the iliac crest at the time of surgery relapsed within 13 months; in contrast, only 36.6% of patients with no evidence of occult metastases in the bone marrow relapsed within the same period ($P < 0.050$) [28]. The presence of occult metastases was most highly associated with skeletal muscle metastases and/or local outgrowth of residual tumor cells.

Studies from our group have also shown that occult

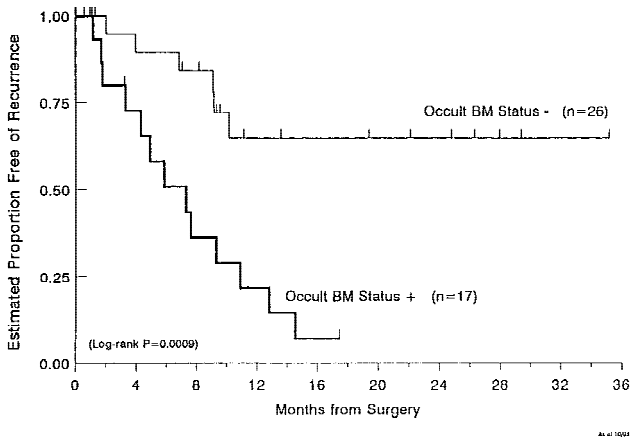

Fig. 2. Recurrence-free interval for patients with stage I to III lung cancer according to bone marrow status. Patients with detectable tumor cells in the bone marrow (thick line) had significantly shorter time to recurrence than patients with no bone marrow occult metastases (thin line), $P = 0.0009$. Reprinted from Cote et al. [24], with permission of Lippincott-Raven Publishers.

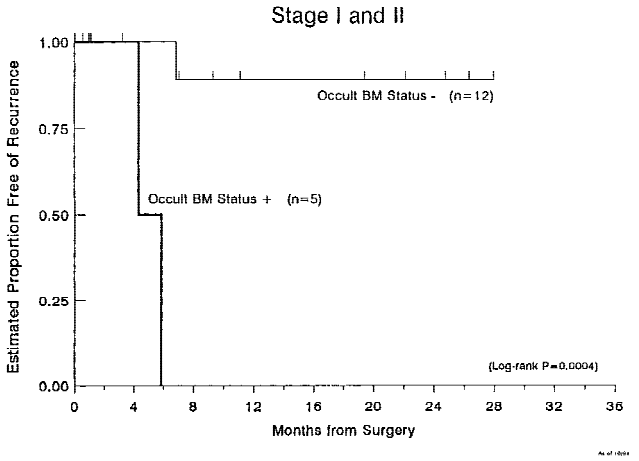

Fig. 3. Recurrence-free interval for 17 patients with stage I or II lung cancer according to bone marrow status. Patients with occult metastases of the bone marrow (occult bone marrow status +, dark line) had significantly shorter time to recurrence than patients with no occult metastases in the bone marrow (occult bone marrow status −, thin line) ($P = 0.0004$). Reprinted from Cote et al. [24], with permission of Lippincott-Raven Publishers.

metastases in the bone marrow can be detected in a substantial proportion of patients with lung cancer who have no clinical evidence of systemic metastases, including patients with disease in its earliest stages [24]. In our study, two cytokeratin-specific monoclonal antibodies, AE-1 and CAM 5.2, were used in combination to detect the presence of tumor cells in the bone marrow of 43 patients with non-small cell cancer. The rate of detection of occult metastases in the bone marrow was again associated with stage of disease: 29% of patients with stage I and II and 46% of patients with stage III disease had detectable occult metastases.

We have shown [24] that patients with occult metastases in the bone marrow had significantly shorter times to disease recurrence compared with patients without occult metastases. The median time to recurrence for patients with no detectable occult metastases was 35.1 months compared with 7.3 months for patients with occult metastases ($P < 0.0009$, Fig. 2). Furthermore, there was a highly significant difference in recurrence rates for patients with stage I and II lung cancer according to bone marrow status ($P < 0.0004$, Fig. 3). Overall survival was also lower in patients with occult metastases: of the 17 patients with occult metastases in the bone marrow, seven (41%) died compared with six (23%) of 26 patients without occult metastases in the bone marrow (Fig. 4).

Similar results were seen for patients with stage III lung cancer, although they did not reach statistical significance ($P = 0.11$, Fig. 5). It was particularly interesting that when occult metastases in the bone marrow were not detected in patients with stage III disease, a substantial proportion (40%) remained disease-free at 2 years, despite the fact that they had more advanced-stage disease.

A multivariate analysis of bone marrow status that controlled for stage of disease showed that the presence of occult metastases in the bone marrow is an independent predictor of disease recurrence [24].

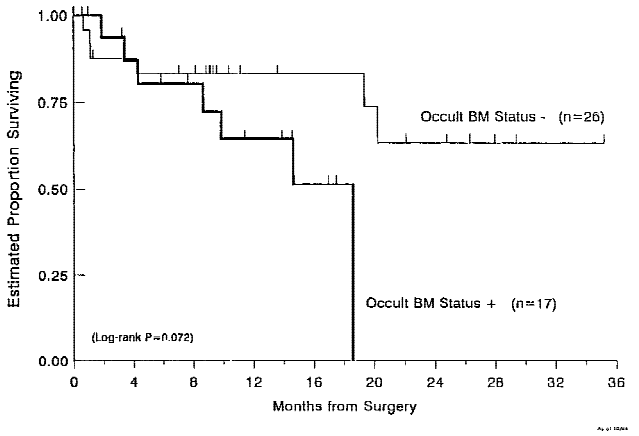

Fig. 4. Survival of 43 patients with stage I to III lung cancer according to bone marrow status. Patients with occult metastases in the bone marrow (occult bone marrow +, dark line) died sooner on average than patients with no occult metastases in the bone marrow (occult bone marrow status −, thin line). This did not reach statistical significance ($P = 0.072$). Reprinted from Cote et al. [24], with permission of Lippincott-Raven Publishers, Inc.

In another study, Pantel et al. [52] demonstrated that occult metastases in the bone marrow could be detected in 83/139 (60%) patients with non-small cell lung cancer without evidence of distant metastases. The presence of occult metastases in the bone marrow was a significant and independent predictor for a later clinical recurrence in node-negative patients ($P = 0.004$), with recurrence seen in 19/54 (35%) patients without detectable occult metastases vs. 9/12 (75%) patients with occult metasta-

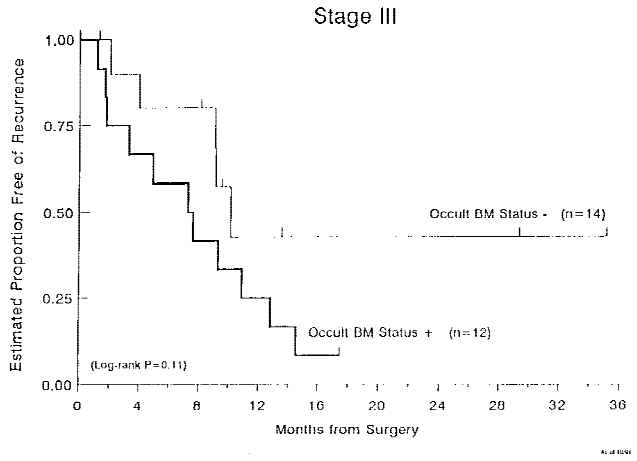

Fig. 5. Recurrence-free interval for 26 patients with locally advanced (stage III) lung cancer according to bone marrow status. Patients with occult metastases in the bone marrow (occult bone marrow status +, dark line) had shorter time to recurrence than patients with no occult metastases in the bone marrow (occult bone marrow status −, thin line). This did not reach significance ($P = 0.11$). Reprinted from Cote et al. [24], with permission of Lippincott-Raven Publishers.

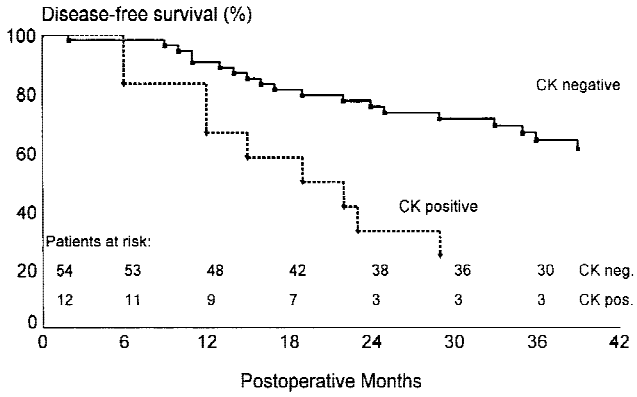

Fig. 6. Cumulative disease-free survival of 66 lymph node-negative patients plotted according to presence or absence of cytokeratin 18, (CK-18) tumor cells in the bone marrow ($P = 0.004$). Reprinted with permission from Pantel R, Izbicki J, Passlick B, et al. Frequency and prognostic significance of isolated tumour cells in bone marrow cells in patients with non-small cell lung cancer without metastases. 347: 649–653. ©The Lancet, Ltd. 1996.

ses. Furthermore, for patients with node-negative disease ($pT_{1-3}pN_0M_0$), those who showed more than one cell per 4×10^5 bone marrow mononuclear cells were significantly more likely to suffer relapse (Fig. 6).

Ohgami et al. [53] compared 39 patients with stage I–III non-small cell lung cancer who underwent curative resection using IHC with monoclonal antibody to CK-18 to detect tumor cells in the bone marrow. They also performed immunostaining of p53 protein in the corresponding primary tumors. CK-18-positive cells were detected in 15 (39%) of the 39 patients and overexpression of p53 was associated with positivity of the tumor cells in the bone marrow. Also, patients with CK-18-positive cells in the bone marrow demonstrated a significantly earlier recurrence than patients without detectable occult metastases in the bone marrow.

Thus, evidence is accumulating that the presence of occult metastases in the bone marrow is a clinically significant event in patients with non-small cell lung carcinoma (Table I). The detection of occult metastases may be a way to identify patients with early-stage disease who would most benefit from adjuvant systemic treatment. At the very least, occult bone marrow metastasis detection should be incorporated into future clinical trials.

Small Cell Lung Cancer

Small cell lung cancer remains a challenge to surgeons, radiotherapists, and medical oncologists because of its very high propensity to metastasize to distant sites. Although combination chemotherapy has been shown to affect survival, remission is only temporary and successive relapses become increasingly resistant to therapy [48]. Leonard et al. [54] have shown that detection of occult metastases in the bone marrow in small cell lung cancer patients predicts metastatic relapse. This study examined bone marrow from 12 patients in clinical remission from small cell lung cancer. Using IHC, metastatic deposits of tumor cells were found in the marrow of eight (67%) of these 12 patients. Six of these eight IHC-positive patients were determined to have marrow involvement at the time of restaging and subsequently suffered metastatic relapse. The four patients established as negative for tumor presence by IHC did not suffer metastatic relapse.

These results indicate that the detection of occult metastases in small cell lung cancer can predict progression and might have significant implications for the choice of therapy. In fact, the results suggest that use of this method to detect occult metastases in bone marrow can identify a subset of patients with small cell lung carcinoma (those with no detectable tumor cells in bone marrow) who may benefit from a primary surgical approach (lobectomy) in the treatment of their tumors. This would represent a substantial and important departure from current therapeutic recommendations.

DETECTION OF OCCULT METASTASES IN REGIONAL LYMPH NODES OF PATIENTS WITH LUNG CANCER

Despite the explosion in our knowledge of the biology of cancer, the single most important prognostic factor for most solid tumors that do not have evidence of systemic dissemination is the presence or absence of regional lymph node metastases. As we have seen, systemic dissemination may take place by routes other than lymphatic spread; the presence of occult metastases in the bone marrow in node-negative patients demonstrates this. It has now become clear that another possible site

TABLE II. Median Survival in Patients With and Without Occult Regional Lymph Node Metastases

Group	Total number patients	No. occult LN-positive	Median follow-up	Median overall survival		*P* value
				LN-negative	LN-positive	
Chen et al. [40]	60	38 (63%)	Not available	81.9 months	65.9 months	$0.10 > P < 0.05$
Passlick et al. [29]	66	10 (15%)	26 months	Not available	Not available	$P = 0.005$
Izbicki et al. [39]	67	16 (24%)	Not available	44.6 months	36 months	$P = 0.008$

for occult tumor spread in node-negative patients is the regional lymph nodes.

Routine histopathologic examination of lymph nodes is in reality only a sampling of the lymph node. For example, Gusterson and Ott [34] have calculated that a pathologist has only a 1% chance of identifying a metastatic focus of cancer with a cross-sectional diameter of three cells within a lymph node. In addition, reexamination of lymph node sections initially considered ''negative for tumor'' after routine histologic screening frequently show metastatic tumor deposits, demonstrating that even when tumor cells are present in the section they can be missed. It is evident that routine processing and examination of lymph nodes is inadequate for detecting the presence of tumor in all cases.

Chen et al. [40] used a polyclonal and anticytokeratin antibody to examine regional lymph nodes from 60 patients with node-negative non-small cell lung cancer. They demonstrated that regional lymph nodes contain metastatic tumor cells at a much higher frequency than was previously determined by conventional histologic methods. Of the 60 patients examined in this study, 38 (63%) were shown to have occult metastases. The median survival of patients with occult nodal metastases (1,977 days) was shorter than that of patients whose lymph nodes showed no tumor cells (2,456 days).

Passlick et al. [29] used the antiepithelial cell antibody Ber-Ep-4 against epithelial cells to study regional lymph nodes from 72 patients with node-negative non-small cell lung cancer. Eleven of the 72 (15.2%) were found to have positive staining. The presence of occult tumor deposits in lymph nodes was significantly associated with a shorter disease-free survival. After a median follow-up of 26 months, five of 10 patients (50%) with occult nodal metastases had recurrences, as compared with eight of 56 (14.2%) of those without nodal metastases (Table II).

Izbicki et al. [39] studied patients with non-small cell lung cancer for the presence of occult metastases in the lymph nodes using IHC. They found that of 67 patients available for follow-up with nodal stage N0, 51 patients had no evidence of occult metastases using antibody Ber-Ep-4 in an IHC assay; the mean relapse-free survival and cancer-related survival rates for this group were 41.1 months and 44.6 months, respectively. In contrast, for the 16 patients with nodal occult metastases, the mean relapse-free survival and cancer-related survival rates were 29.0 months and 36.5 months, respectively. The difference in relapse-free and cancer-related survival between the two groups (occult nodal metastases status negative vs. positive) was significant ($P = 0.0081$ for relapse-free survival, $P = 0.0584$ for cancer-related survival) (Fig. 7). In this study, they also compared the size of occult nodal metastases. Using a cut-off of three of fewer disseminated cells and more than three cells, they showed no statistically significant differences with respect to relapse-free and cancer-related survival rates and distant metastasis-free and local recurrence-free intervals (Fig. 8). This indicates that the detection of even a single epithelial cell in a lymph node may be a marker for disseminated disease. They also noted that a comparison between patients with disease staged as N0 or N1 with nodal occult metastases and a control population of 36 patients with proven N2 stage disease revealed no statistical difference in cancer-related survival (Fig. 9), indicating that the presence of occult nodal metastases has a similar effect on recurrence as overt nodal metastases.

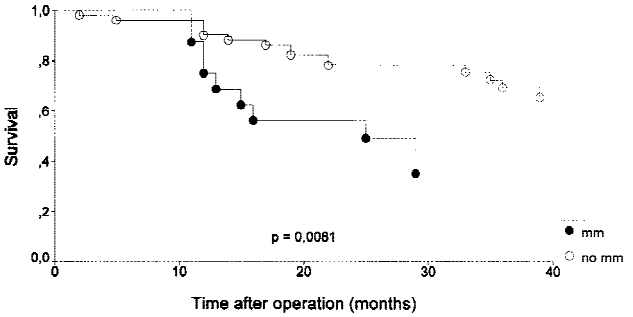

Fig. 7. Relapse-free survival curves among patients with non-small cell lung carcinoma plotted according to occult lymph node metastases (mm) status (22 patients with positive lymph nodes, 62 without positive lymph nodes) for stage pT1 to pT4pN0 ($P = 0.0081$). Reprinted with permission from Izbicki JR, Passlick B, Hosch SB, et al. Mode of spread in the early phase of lymphatic metastases in non-small-cell lung cancer: significance of nodal micrometastases. J Thorac Cardiovasc Surg 1996;112:623–630.

Thus, it is becoming clear that the presence of occult nodal metastases is a powerful predictor of outcome in patients with non-small cell lung cancer.

MODIFICATIONS OF THE TNM CLASSIFICATION SYSTEM

The ability to detect occult systemic and regional dissemination of tumor has revolutionized our approach to

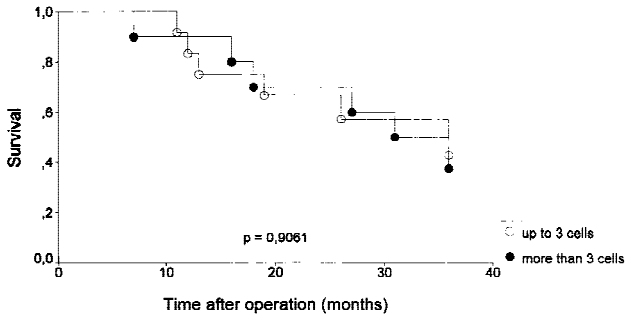

Fig. 8. Cancer-related survival curves for patients with non-small cell carcinoma of the lung (T1–T4, N0–N1) with three or fewer disseminated cells (n = 12) and with more than three disseminated cells (n = 10, does not reach statistical significance). Reprinted with permission from Izbicki JR, Passlick B, Hosch SB, et al. Mode of spread in the early phase of lymphatic metastases in non-small-cell lung cancer: significance of nodal micrometastases. J Thorac Cardiovasc Surg 1996;112:623–630.

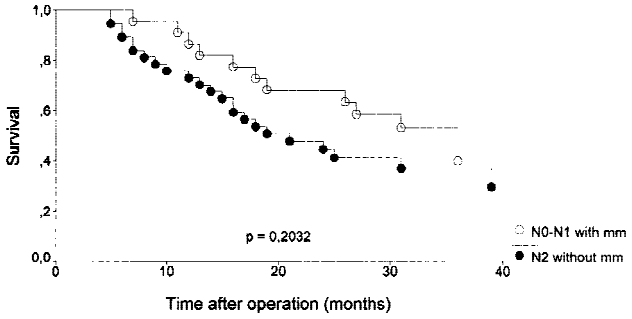

Fig. 9. Cancer-related survival curves for patients with pT1–pT4pN0–pN1 non-small cell carcinoma of the lung with occult metastases (N0–N1 with mm, n = 22), and patients with pT1–pT4pN2 disease without occult metastases (N2 without mm, n = 36, does not reach statistical significance). Reprinted with permission from Izbicki JR, Passlick B, Hosch SB, et al. Mode of spread in the early phase of lymphatic metastases in non-small-cell lung cancer: significance of nodal micrometastases. J Thorac Cardiovasc Surg 1996;112:623–630.

tumor staging [48]. That the detection of occult metastases is prognostically and biologically important for a wide variety of tumors is being reinforced with each new carefully performed study, and this is now well shown for lung carcinoma. Because of the general application and prognostic importance of these findings and because the presence of occult metastases can specifically influence therapeutic approaches, it may be important to include information about occult metastases in the staging of tumors. We therefore suggest modification of the TNM staging system to include lymph node and bone marrow occult metastasis status: TNnMm, with *n* and *m* being the occult metastatic status of the regional lymph nodes and bone marrow, respectively. If implemented, this should represent a significant advance in the individual evaluation and treatment of patients with cancer. At the very least, examination for occult metastases should be incorporated into future clinical trials evaluating treatment approaches to lung cancer.

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